NIOSH
cg98: expedited process

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Date: December 1, 2010

TO: Katherine Norris **Fax No.** 404-498-1575

of: CDC/ATSDR FOIA Office **Tel No.** 404-498-1580

FROM: Marlene A. Moore **Direct Dial No.** (614) 464-8309

Direct Fax No. (614) 719-4878

Number of pages (including this sheet): 3 (incl.

Dear Ms. Norris,

Please find attached a FOIA Request for documents in the possession of the National Institute of Occupational Safety and Health which were obtained during their investigation of the Goodyear St. Mary's Plant, St. Mary's Ohio.

Should you have any questions with regard to our request, please do not hesitate to contact me at your earliest convenience at 614-464-8309. We hope to be able to expedite our request and will cooperate by answering your questions or any matters of concern in a timely manner.

Marlene A. Moore
Paralegal

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8309	055429	000034

If you have any problems with transmission, please call (614) 464-8309

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December 1, 2010

Center for Disease Control and Prevention
CDC/ATSDR/FOIA Office
ATTN: Katherine Norris, FOIA Office
MSD 54
1600 Clifton Road, NE
Atlanta, GA 30333

Dear Ms. Norris:

Pursuant to the Freedom of Information Act, 5 U.S.C. subsection 552, I am hereby requesting all records and documents of any kind (including all records, information, or data stored on microfilm) in the possession of the National Institute of Occupational Safety and Health ("NIOSH") which were obtained, referenced, used, created, or assembled during the NIOSH investigation of the Goodyear Tire & Rubber Company's St. Mary's, Ohio Plant Pliofilm Department ("St. Mary's"), including, specifically the documents obtained during the 1970s studies conducted by NIOSH and its representatives. This request includes any and all documents (including microfilm copies) utilized by NIOSH for their studies of the St. Mary's Plant, including all sampling, monitoring, or process data and all documents listing or referencing any names or identifying information for the St. Mary's Plant employees that were blood tested on a regular basis during the relevant time period. The request also includes memoranda, reports, correspondence, summaries, analyses and notes prepared by NIOSH representatives.

VORYS
Legal Counsel

We also request a copy of any and all notes taken by NIOSH representatives during meetings with representatives from the Goodyear Corporation and all notes taken during visits to the St. Mary's Plant.

If there are any fees for searching for, reviewing, or copying the records, please notify me before processing if the amount exceeds $2,500.00. To the extent that the records we are requesting are stored on microfilm, please advise as to whether representatives from our law firm are permitted to review the data at your facility.

If you deny all or any part of this request, please cite each specific exemption that you think justifies your refusal to release the information and notify me of appeal procedures available under the law.

If you have any questions with regard to the purpose of this request, you may telephone me at (202) 467-8811 or you may telephone Ms. Marlene Moore, a paralegal at the Vorys firm who is conducting our assembly of records, at (614) 464-8309. Please also advise Ms. Moore by email at: mamoore@vorys.com or at her office number when the documents or microfilm are ready for review.

Very truly yours,

Joseph D. Lonardo

Joseph D. Lonardo

JDL/rbj
cc: Robert C. Mitchell, Esq.
F. Daniel Balmert, Esq.
Marlene Moore

Dr. Wagoner
61.2

UNI) STATES ENVIRONMENTAL PROT TION AGENCY
kesearch Triangle Park, North Carolina 27711

SUBJECT: EPA Interest in NIOSH Benzene Studies DATE: 29 OCT 1976

FROM: Richard J. Johnson, Program Analyst RJJ
Strategies and Air Standards Division - EPA

TO: Richard Hartle, Industrial Hygienist
Surveillance, Hazard Evaluation, and Field
Studies Division - NIOSH

As we discussed, several months ago our division identified benzene, from among twenty-two high volume industrial chemicals which were evaluated as potential air pollution problems, as a chemical requiring a more detailed effort. The enclosed memo addresses some of the reasons why benzene was selected and details a work plan for obtaining additional information information on benzene. In general, we are interested in your division's benzene investigations because we are concerned about the potential problem to community health from exposure to ambient benzene and the eventual need to regulate sources of atmospheric benzene emissions. Your Division's epidemiologic studies into blood dyscrasias resulting from occupational exposure to benzene may be of importance to EPA's efforts in better understanding the significance of atmospheric benzene. Also, results from your Division's service station attendant exposure study may be helpful to EPA in evaluating the importance of community exposure to benzene from evaporative losses at service stations. This source is one of several whose emissions may pose a potential problem to community health.

Recently your agency recommended that benzene be labeled a "human carcinogen" and recommended to the Department of Labor that the current eight-hour occupational exposure standard for benzene be lowered by a factor of ten. This recommendation and some preliminary monitoring results for atmospheric benzene, have heightened EPA's interest in the significance of ambient benzene.

With these reasons in mind, I would appreciate NIOSH's support in keeping EPA informed of the studies indicated above as well as any other benzene related activities. The information you have provided has been very helpful to our efforts. Thank you for the cooperation you have shown me thus far. I trust that some of my comments regarding additional statistical analysis of your Division's service station attendant study have been useful.

I look forward to our meeting on November 16.

Enclosure

cc: John O'Connor
Joseph Padgett

Research Triangle Park, North Carolina 27711

Benzene Action Plan

July 22, 1976

Richard Johnson, Program Analyst
Pollutant Strategies Section

Joseph Padgett, Director
Strategies and Air Standards Division

In response to a growing concern for benzene's potential as a carcinogen, a number of Federal Agencies have recently initiated programs for obtaining additional information on benzene. In line with these activities, the Pollutant Strategies Section has begun a program to evaluate benzene as a potential community air pollution problem. The purpose of this memo is to outline the activities of other Federal Agencies: review our current knowledge regarding the health effects, sources of benzene and air quality levels; and propose a program of study aimed at assessing the community air pollution significance of benzene.

Activities of Other Agencies

The Occupational Safety and Health Administration (OSHA) has recently initiated a review of the current occupational exposure standards for benzene. These standards*, which were recommended by the National Institute of Occupational Safety and Health (NIOSH) in 1974, are being reassessed as a result of increasing evidence and public reaction to this evidence which indicates chronic exposures to benzene can predispose humans to leukemia.

In reviewing these standards OSHA will make a decision, probably within six months, on whether or not to label benzene as a carcinogen. Should OSHA decide to label benzene as a carcinogen, then minimum feasible occupational standards will likely be proposed. The levels of these standards would be determined in part by results from the Inflationary Impact Statement. This economic analysis, which is being prepared under contract by A.D. Little, Inc, will likely be available for public comment by late fall of this year.

As a part of this review, NIOSH has recently initiated studies to gain additional intelligence on worker exposures to benzene. Perhaps the most important of NIOSH's activities are their soon to be completed investigation into service station attendant exposures to benzene and their recently initiated investigation into blood abnormalities of

*10 ppm as an 8 hour time weight average
25 ppm as a one hour average
50 ppm excursion limit

certain industrial workers. Their service station attendant exposure study, which will probably be completed by early September, is an attempt to estimate service station attendant exposure levels to benzene and certain other pollutants (toluene, xylene, ethylene dibromide, tetraethele lead, carbon monoxide). Their blood abnormalities study, which was recently initiated at a plant manufacturing artifical rubber, involves about a one month effort of simultaneously monitoring benzene concentrations in the plant and drawing blood samples from a number of employees who work in key areas in the plant.

Health Effects

Numerous clinical investigations, using experimental animals, have been conducted to gain an understanding of potential adverse effects from both acute and chronic benzene exposure. Several case histories have been documented of human occupational exposure effects to benzene. Currently, no epidemiologic studies are known to exist which evaluate ambient atmospheric benzene exposure effects in humans.

The most unique clinical aspect of chronic benzene inhalation is hematopoietic (blood) system damage. Anemia, characterized by decreased red blood cell count; thronbocytopenia, a reduction in platelet numbers; leukopenia, a depression of white blood cell count; and low hemoglobin concentrations are often exhibited in patients suffering from chronic benzene inhalation.

The carcinogenic potential of chronic benzene exposure cannot be conclusively established. Numerous articles have appeared in newspapers which linked occupational benzene exposure to leukemia induction in humans. Significant chromosome aberrations have been shown to result from chronic benzene inhalation in both humans and other mammals. These aberrations, which were still present several years after cessation of exposure, may be indicative of leukemia induction since chromosome alterations have been reported in patients with a history of benzene exposure followed by diagnoses of leukemia. However, no conclusive evidence appears to exist from the numerous carcinogenic investigations which have been conducted. That these investigations, using experimental animals, have not produced conclusive evidence, may be due to a number of factors; lack of proper experimental controls, statistically non-significant data, or parallel occurrence of leukemia in control groups.

In addition to these considerations, because benzene's potential as a human carcinogen has been established through case studies only and because experiments using animals have not conclusively established carcinogenicity, other factors have been suggested to explain this apparent lack of consistent findings. Since case studies are not controlled experiments, the probability is high that other air pollutants were present in the occupational environments, which could

have combined with benzene to produce a carcinogenic mixture (synergistic effect). Some animal studies, involving benzol (benzene, toleune, and xylene), exist which support this hypothesis. Second, there exists the possibility that benzene may be a human carcinogen and not also produce leukemia in animals. A number of physicological reasons could be advanced to support this possibility. Perhaps the fact that the amino acid structure of man, which is different from any other mammal, may be one of the more important reasons.

Sources and Emissions

The major class of benzene emissions are most likely those manmade sources which emit benzene directly (primary sources) to the atmosphere. While it is doubtful that there exists any significant natural sources of benzene, some questions exist as to whether or not secondary formation--the degradation or reaction of other compounds in the atmsophere to form benzene--is a significant source of ambient benzene levels.

The major categories of primary manmade benzene are: gasoline evaporation, automotive sources, benzene production, benzene consumption, and coke oven operations. Precise emission estimates of the annual levels of each of these source categories are not known. In the "Air Pollution Assessment of Benzene" by the Mitre Corporation, estimates have been made of the 1971 emission levels (Table 1) for each of these source categories.

Table 1

Estimates of 1971 Benzene Emissions

Source Category	Emissions ($\times 10^6$ lbs/year)	Percent Contribution
Automotive Sources	940	50
Benzene Consumption	658	35
Coke Oven Operations	122	7
Benzene Production	89	5
Gasoline Evaporation	53	3
TOTAL	1862	100

The estimate for the largest of these--automative exhaust--has probably experienced the greatest change. With the increasing use of the catalytic converter, considerable decrease could be expected in benzene emissions from automobiles; however, as a result of gasoline reformulation due to lead phase down, some increase may again occur

in benzene emissions. Better information is needed on the current and anticipated levels of benzene emissions from automobiles and mobile sources in general.

In addition to the need for more accurate data regarding automotive emissions, additional information is needed to gain a better understanding of the current and future impact of other sources of benzene emissions. With the exception of automotive exhaust and gasoline evaporation, most of the emissions from the other categories are generally located in fairly specific regions of the U. S.

With respect to benzene consumption, which accounts for roughly 35% of the estimated total 1971 benzene emissions, some data are currently available. All of these emissions are postulated to occur at chemical manufacturing plants, where benzene is used as an intermediate in the production of other chemicals. In the Mitre report on Benzene, "upper limit" (probable maximum) estimates are presented of the amount of benzene loss during the manufacture of the largest nine chemicals, which use benzene as an intermediate (Table 2).

Table 2

Upper Limit of the Amount of Benzene Lost From Byproduct Manufacturing Facilities in 1971
(Benzene Consumption)

ByProduct	Benzene Consumption (10^6 lbs)	(% of Total)	100% Minus % Yield*	Amount of Benzene Unaccounted for (10^6 lbs)
Ethylbenzene	3709	44	3	111
Phenol	1610	19	18	290
Cyclohexane	1311	15	0	0
Maleic Anhydride	325	4	43	140
Detergent Alkylate	323	4	20	65
Aniline	297	3	7	21
Dichlorobenzene	94	1	15	14
DDT	43	1	40	17
Other Nonfuel Uses	676	8	--	--
				658

Source: Benzene Environmental Sources of Contamination, Ambient Levels, and Fate, Life Sciences Division, Syracuse University Research Corporation, 1974.

*The yields used are for overall yields to the various by-products. Some of these processes (e.g., phenol) are more than one step and, therefore, the losses may be not only benzene but also the intermediate compounds (e.g., chlorobenzene or cumene with phenol production).

As can be seen from the table, over 40% of the benzene loss from these facilities occurs at phenol manufacturing plants. At least 20% of benzene is lost in the manufacture of maleic anhydride, which was recently assessed as a potential air pollution problem under our contract with GCA. More detailed information is needed regarding levels of benzene emissions at specific manufacturing sites. Information is needed on the current levels of control at these sites as well as feasible levels of control using existing technology. Data are also needed on the growth trends of the production of these chemicals as well as some estimates of future shifts in the distribution of these manufacturing plants.

Coke oven operations, which constitute an estimated 7% of the total 1971 benzene emissions, are fairly specifically located. Most of these operations are located in Alabama, Illinois, Indiana, Ohio and Pennsylvania. Because the method used to develop this emission estimate is crude, better data are needed on benzene emissions from coke ovens. Information is needed regarding the levels of benzene emitted from coke oven operations employing different types of processes and controls as well as geographic locations of these operations. These specific estimates can input to diffusion modelling to provide estimates of ambient benzene levels in the vicinity of specific coke oven operations.

Benzene production facilities, which amounted to about 5% of the 1971 total estimated benzene emissions, are concentrated to some extent in Texas and Louisana. About 56% of the benzene emitted from benzene production occurrs in Texas and Louisiana with over 50% of the benzene production facilities also located in these two states. About 40% of the 1971 total benzene emissions occurred in Texas alone, with most of these emissions occurring in three counties; Jefferson, Harris, and Nueces. In addition to these considerations, about 25% of the national total of benzene emitted from the production of benzene occurred at the five largest benzene production facilities, four of which are located in Texas and one located in Louisana. Air quality data and (or) dispersion modelling data are needed in the vicinity of these facilities and perhaps in several locations in each of the three counties in Texas. Emissions testing about some of the largest plants are also suggested. Information on current levels of control and feasible levels of control using current technology are needed.

Benzene emissions from gasoline evaporation, which constitutes an estimates 3% of the total 1971 benzene emission, are probably distributed uniformly on national basis with population density. Recent information indicate that the benzene content in gasoline, while varying somewhat both ¢ regionally and ¢ seasonally, is roughly 3% by volume. With the scheduled implementation of gasoline marketing controls in some 15 AQCR's, benzene emissions from gasoline evaporation will likely be considerably reduced in these AQCR's. In addition to these considerations, the NIOSH service station attendant exposure investigations will provide some insight regarding ambient benzene levels about gasoline stations. This information will be useful in evaluating the significance of service stations as sources of benzene emissions.

Ambient Levels

Although little data are available on ambient benzene concentrations, considerable data are becoming available which confirm the presence of benzene at most sampling sites. Qualitiatively, benzene has been detected in the atmosphere of most sites sampled throughout the United States. ORD, through a contract with Research Triangle Institute (RTI), has been and will continue to sample qualitatively for benzene in many urban and industrial atmospheres throughout the U. S. ORD has recently begun to sample quantitatively for benzene. Some of the quantitative data, not yet validated, are presented in Table 3.

Table 3

Location	Concentration (ppm)**
Edison, New Jersey* (0.25 miles downwind)	0.43
Edison, New Jersey (1 mile upwind)	0.06
Edison, New Jersey (within dump area)	0.28
Edison, New Jersey (upwind)	trace
Edison, New Jersey	0.004
Belle, West Virginia	0.06-0.28

*At a land fill where wastes from chemical processes are being dumped.

**Averaging time of roughly one-half to two hours

Proposed Program

In the previous paragraphs, activities of other federal agencies concerning benzene were discussed, available information necessary to evaluating atmospheric benzene as a potential air pollution problem were also discussed, as well as gaps in the current body of information. In the following paragraphs a program with estimates of resource requirements is proposed to fill these information gaps; thus, providing a reasonably complete database to assess benzene as a potential air pollution problem.

1. Follow the activities of other federal agencies.

Maintain close contact with key OSHA personnel who are involved in re-evaluating the current OSHA standards and requirements of this standard (new standards likely to be proposed by December). Track closely the NIOSH investigations currently underway. When data are available from both NIOSH health studies and their investigation of service station

attendant exposures to benzene, probably by mid-September, arrange a mutual meeting with NIOSH personnel and OSHA staff involved in each of these investigations. Write-up summaries of these activities as they occur. Integrate key elements of these summaries into overall benzene assessment paper. 40 manhours

2. Work with OTS to develop a database for evaluating potential population exposure effects of atmospheric benzene.

Through OTS's contract work with System Sciences Incorporated (SSI), who are developing a database to investigate relationships between disease specific mortality rates and community exposures to industrial effluents and emissions, investigate the possible relationship between blood disease mortalities, which chronic benzene exposure can induce, and those U. S. countries where benzene emissions would be expected to be highest (i.e., countries where large benzene production/consumption facilities or numerous coke oven operations are located). This work could be done by SSI staff. The approvals have been cleared for SSI to develop this information. However, because the methodology used to develop this information could be adopted to other pollutants for which the Pollutant Strategies Section may have some future concern, by using in-house personnel, in-house expertise could be developed which could be applied to other pollutants. To follow this approach would require a thorough knowledge of the SSI database (which would probably require a trip to SSI), access to the SSI database (which should be completed and debugged by mid-September), and computer time.

Because of the potential for future use of the SSI database and the possibility of integrating this database with other databases such as NEDS and certain toxicity databases currently being developed, the approach of using Pollutant Strategies staff is recommended over the use of SSI personnel. The benzene analysis including write-up would likely take about two weeks and would probably be able to be started around mid-September. 80 manhours

3. Better characterization of benzene emissions.

Additional information are needed to better understand the sources of benzene emissions. This information, which could be obtained on contract, through a task order, would include reasonably accurate current emission estimates for the various specific sources of benzene emissions, current levels of control of benzene emissions about these sources, feasible level of control for these sources, growth trends, and geographic locations and geographic shifts. This work can be initiated, once the SASD contract is awarded - perhaps July 31.

320 manhours (contract)

In addition to this data, information is needed on the amount of ambient benzene which results from secondary formation. Data are needed on emissions of compounds which degrade or react in the atmosphere to

form benzene. Also, some efforts should be devoted to determining if natural resources are a significant source of ambient benzene. This work could be done through in-house staff communicating with scientists who are experts in this field and could be initiated immediately.
32 manhours

4. Obtain additional air quality data.

The current database of ambient benzene concentrations is limited. Additional data are being obtained by ORD both through their Environmental Sciences Research Laboratory (ESRL) and their Industrial Environmental Research Laboratory (IERL). Through contracts which both of these laboratories currently have, they can obtain additional ambient benzene concentrations. Close contact should be established with laboratory personnel involved in each of these contracts toward providing input in planning future monitoring locations. Some preliminary contacts have already been established with key personnel in each of these laboratories.

In addition to these efforts, OTS is in the process of developing a list of sites to monitor benzene. Through a contract OTS has with Battelle, ambient benzene concentrations will be obtained about some 20 to 30 designated sites. SASD has been requested to provide input to selection of these sites. Some input with respect to benzene production facilities has already been provided. 60 manhours

5. Preliminary Air Pollution Assessment Report

Once information has been obtained from each of the above program elements, a thorough assessment, which would evaluate the potential for benzene as an air pollution problem, can be made. 120 manhours

Enclosure

cc: J. O'Connor
M. Jones

SASD:SCAB:PSS:RJohnson:taa:rm947:NCMu:x501:7/22/76

Proposed Tasks for Obtaining Information

to Assess Benzene as an Air Pollution Problem

	Date (Start Complete)
Maintain contact with OSHA personnel	(now - 1/31/77)
Track closely NIOSH investigations	(now - 10/1/76)
Characterize benzene emissions	(8/15/76 - 10/15/75
Work with OTS to develop health effects database for ambient benzene exposure	(9/15/76 - 9/30/76)
Meet with NIOSH and OSHA personnel to discuss data from NIOSH investigation	(about mid-October)
Obtain additional air quality data	(continuing)
Write preliminary air pollution assessment report	(11/15/76 - 12/10/7

External Review Draft
October 1977

BENZENE HEALTH EFFECTS ASSESSMENT

NOTICE

This document is a preliminary draft. It has been released by EPA for public review and comment and does not necessarily represent Agency policy.

U.S. ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF RESEARCH AND DEVELOPMENT
WASHINGTON, D.C. 20406

External Review Draft
October 1977

BENZENE HEALTH EFFECTS ASSESSMENT

NOTICE

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U.S. Environmental Protection Agency
Office of Research and Development
Washington, D.C. 20460

CONTENTS

ACKNOWLEDGMENTS

This document was prepared by EPA's Office of Research and Development with extensive help from a team of consultants led by Bernard D. Goldstein, M.D. Major contributions were by Carroll A. Snyder, Ph.D., Robert Snyder, Ph.D., and Sandra R. Wolman, M.D. The views represented in this document are those of the EPA and not necessarily those of the consultants.

The final document will in addition incorporate, as appropriate, comments and contributions from many sources, and especially those from EPA's Scientific Advisory Board.

SUMMARY AND CONCLUSIONS

This report presents the research findings on benzene toxicity relevant for assessing human health risks at environmental exposure levels. The principal conclusions to be drawn from this report are:

1. Benzene exposure by inhalation and other exposure routes is strongly implicated in three pathological conditions that may be of public health concern at environmental exposure levels:

1) leukemia, especially acute myelogenous leukemia

2) pancytopenia (including aplastic anemia)

3) chromosomal aberrations.

2. The epidemiological data, from occupational exposure studies, argue convincingly that benzene is a human leukemogen. The exposure data in these studies do not allow a scientific derivation of a dose-response curve. Most studies in which exposure levels were determined involved exposures in the range of 100 to 500 ppm, though in some the benzene concentrations were lower.

3. The data do not indicate that any population segment is particularly susceptible.

4. Currently there is no convincing evidence that benzene causes neoplasias, including leukemia, in animals. Failure to induce leukemia in animals could be due to an as-yet-unknown cocarcinogen required to evoke the leukemogenic response initiated by benzene.

5. Hematotoxicity, particularly pancytopenia, has been observed in both humans and animals, following exposure to benzene. The toxicity does not follow exposure to other compounds such as toluene and xylene commonly associated with benzene environmentally.

6. Humans who develop hematologic abnormalities due to benzene exposure have a greatly increased probability of developing leukemia and aplastic anemia, a finding consistent with the thesis that benzene is leukemogenic.

7. Long-term occupational exposures of workers to benzene at levels as low as 20 ppm but generally at levels greater than 100 ppm have resulted in various signs of hematotoxicity.

8. Two effects, as yet unconfirmed, of potential significance have been reported at occupational exposure levels of 3 to 15 ppm. The effects are 1) an increase in red blood cell levels of deltaaminolevulinic acid, a precursor in the meme biosynthetic pathway, and 2) a decrease in the mean serum complement of the blood.

9. Available data from studies where measurements ranged from 25 to 150 ppm strongly suggest that chromosome breakage and rearrangement can result from chronic exposure to benzene. These aberrations have been observed to persist in lymphoid and hematopoietic cells after removal from benzene exposure. Since a favored mechanism for leukemia development is somatic mutation, the persistence of chromosomal aberrations, coupled with clinical observations of chromosomal abnormalities in human leukemic cells, support the thesis that benzene is a leukemogen. A dose-response relationship has not been demonstrated for benzene-induced chromosome aberrations. This lack may result from variations in individual susceptibility.

10. Benzene toxicity probably occurs via a toxic metabolite.

11. In animals, benzene accumulates in lipoid tissue such as fat and bone marrow, and benzene metabolites concentrate in the liver and bone marrow. The concentration of metabolites in the bone marrow exceeds that in the blood.

12. The accumulation of benzene metabolites in bone marrow along with the coincidental covalent bonding of benzene to solid residues of bone marrow is consistent with a phenomenon of toxico- and carcinogenesis shared by many other chemicals.

SECTION 1

INTRODUCTION

There is substantial evidence that concentrations of benzene encountered in the work place (in the United States and elsewhere) have caused diseases of the blood and bone marrow in general (e.g., blood dyscrasia, pancytopenia) and leukemia in particular (especially acute myelogenous leukemia). Because current policy of the Environmental Protection Agency (EPA) states that there is no zero risk level for carcinogens, benzene has been listed by EPA under Section 112 of the Clean Air Act as a hazardous air pollutant.

As an aid in determining what regulatory action (if any) should be taken by EPA on benzene, three reports have been prepared:

1. A health effects assessment,
2. An environmental exposure assessment, and
3. A risk assessment based on the data in the first two assessments.

This report is the health effects assessment; it is largely a review and evaluation of the scientific literature relevant to determining the human health effects of environ-

mental exposures to benzene. Most of what is known concerning the effects of benzene on human health has been learned by studies of persons exposed to benzene in the workplace. Virtually no information is available that describes the health effects of nonoccupational exposures of the general populace to benzene. Our evaluation of potential environmental health effects, then, must be based upon what we know of the mechanisms of benzene toxicity and its genetic implications and of the effects of benzene on animals and on human beings. This report is structured accordingly.

Section 2 introduces some of the major biomedical concepts that are pertinent to assessment of the health effects of benzene. Following a brief discussion of benzene metabolism in animals and in humans, the cytogenetic effects of benzene are considered, particularly its effects on chromosomes.

The major portion of the report deals with assessments of benzene toxicity in animals (Section 3) and in man (Section 4). These latter analyses focus on two forms of benzene-induced disorders: 1) pancytopenia, defined as the diminution of all formed elements in the blood, and 2) leukemia, defined as a proliferation and accumulation of mature and immature white blood cells (leukocytes) in blood and/or in bone marrow, leading to the impairment of normal function.

SECTION 2

BENZENE METABOLISM AND CYTOGENETIC EFFECTS

BENZENE METABOLISM

Metabolism in Animals

Most of the benzene that enters the body is excreted via the lungs in exhaled air.[3,42] Study of the distribution of benzene and its metabolites in animal organs shows that free benzene accumulates in lipoid tissue such as fat and bone marrow. High concentrations of benzene metabolites can be observed in liver tissue and in bone marrow. It is particularly significant that the concentration of metabolites in bone marrow exceeds that in blood.[3] Repetitive administration of benzene leads to accumulation of both benzene and its metabolites in these organs and to covalent binding of benzene metabolites to liver and to solid residues in bone marrow.[53]

The metabolic pathway of benzene in liver is shown in Figure 1.[54] The initial step appears to be a reaction mediated by the mixed-function oxidase.[13] This enzyme is inducible, so that pretreatment with benzene, phenobarbital, or 3-methylcholanthrene can increase the rate of benzene

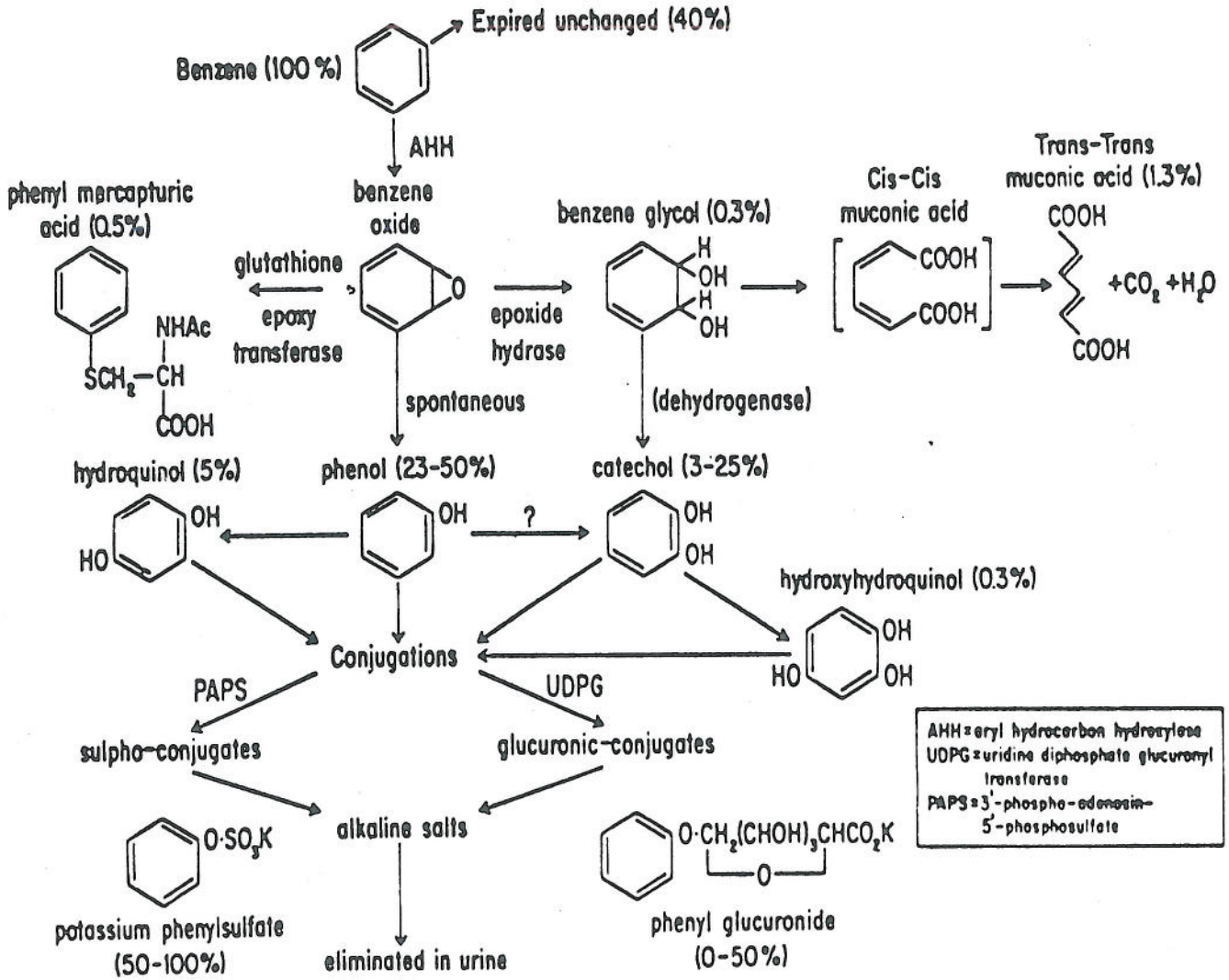

Figure 1. Metabolic pathway of benzene in liver.

metabolism.[13,44,51] The direct product of the interaction of benzene with mixed-function oxidase is probably an arene oxide that is highly reactive. It can spontaneously rearrange to form phenol, undergo enzymatic hydration followed by dehydrogenation to form catechol or a glutathione derivative (phenylmercapturic acid), or bind covalently with cellular macromolecules. Formation of hydroquinone or of trihydroxylated compounds probably is the result of several reactions with hydroxylating enzymes.

Benzene Metabolism in Man

Most metabolic studies of benzene in man have been concerned either with uptake and excretion of unchanged benzene via the breath or with measurements of benzene metabolites in urine. Nomiyama and Nomiyama[40] exposed volunteers to a series of solvent vapors and found that among six subjects exposed to benzene at 52 to 62 ppm for 4 hours, retention of benzene in the respiratory system decreased and then became constant after 3 hours at 30.2 percent of the inspired dose. There was no distinction attributable to sex of the subjects. Excretion, as measured in exhaled air after removing the subject from the benzene-laden atmosphere, was about 16.8 percent. Net uptake, i.e. the sum of uptake and excretion, was 46.9 percent. These

authors[39] went on to show that when the logarithm of the benzene concentration in expired air was plotted against time, the excretion pattern described a hyperbole that could be expressed mathematically and that yielded three rate constants to describe the phenomenon. The subjects continued to excrete benzene in the exhaled air for as long as 15 hours. Hunter,[18] in studies of people exposed to 100 ppm benzene, detected benzene in the expired air 24 hours later. He suggested that measurements of benzene in expired air could be used to estimate benzene content of the inspired air by extrapolation.

Phenol content of the urine is often measured after benzene exposure. Maximum concentrations are thought to occur within 2 hours after exposure.[18] The major conjugated form appears to be ethereal sulfate until phenol levels of the urine reach 400 mg/liter, at which point glucuronide begins to appear.[50]

Teisinger et al,[57] who exposed humans to benzene at 100 ppm for 5 hours, reported that 46 percent of the dose was retained. Of that amount, 61 percent was recovered as phenol, 6.3 percent as catechol, and 2.4 percent as hydroquinone. In these studies the major monohydroxylated metabolite and the two major dihydroxylated metabolites observed by Parke and Williams[42] in rabbits were also observed in man.

Relationship of Benzene Metabolism to Benzene Toxicity

Since Parke and Williams[42] suggested in 1954 that a metabolite of benzene is responsible for benzene toxicity, evidence to support that hypothesis has mounted. Nomiyama[37] demonstrated that inhibition of benzene metabolism protected rats against benzene-induced leukopenia. Andrews[3] reported that when benzene metabolism was inhibited with toluene the subjects were protected against benzene-induced reduction of red cell production. Animals have been protected against benzene toxicity when pretreated with phenobarbital,[10,20] probably because phenobarbital stimulates benzene metabolism in liver, which leads to detoxification and thereby reduces the amount of benzene available for formation of the toxic agent in bone marrow. The specific metabolite that produces benzene toxicity has not yet been identified, but likely candidates are benzene oxide, catechol, and hydroquinone, or the corresponding semiquinones.

The demonstration that reduction of red cell count during benzene treatment is accompanied by accumulation of benzene metabolites in marrow and coincidental covalent binding of benzene to solid residues of marrow[53] suggests a phenomenon in toxico- and carcinogenesis shared by a variety of other chemicals, such as acetaminophen,[23] bromobenzene,[64] hydrazine derivatives,[32] parathion,[35] and many others.[22]

Although further studies are required to prove the hypothesis, it seems likely that benzene, like many other chemicals, exerts its toxicity by formation of a toxic metabolite.

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CYTOGENETIC EFFECTS

Concepts

Mutagens and Carcinogens - Benzene is believed to affect chromosomes, and chromosomal aberrations have been sought as indications of a biologic response to benzene for logical reasons. Somatic mutation has long been accepted as a critical event in the initiation or maintenance of malignant change, although the concept is not unchallenged. Focus on sites of genetic damage is based partly on observations of the prolonged delay from the time of exposure to a carcinogen until the advent of malignancy, such delay being consistent with perpetuation of the original damage in the genetic system. Further, many lines of evidence indicate that most, if not all, carcinogens are mutagens.

Rapid, convenient, accurate, and inexpensive systems for mutagen testing are available for evaluation of point mutations in prokaryotic cells;[1] nevertheless, the assessment of damage to mammalian chromosomes is probably more directly relevant to estimations of human health hazards from mutagens. If a cell shows sufficient chromosome alteration that further cell division is interrupted, then from a reproductive point of view that cell is dead and the damage is toxic. If, on the other hand, the chromosome alteration does not interfere with cell division and can be replicated,

then it constitutes a mutation, a structural change in the genome that presumably alters cell function. Chromosomal breaks, which may be repaired, are not mutational events (in the sense of being heritable). However, each occurrence increases the probability of formation of a structural aberration and therefore of a mutation.

Investigations aimed at evaluating effects of benzene have appropriately concentrated on changes in cell nuclei, metabolism of deoxyribonucleic acid (DNA), cell division, and chromosome alterations. All these constitute direct measures of changes in quantity, structure, organization, or function of the cellular DNA. Moreover, some of these changes are heritable and imply permanent changes in the genome of the affected cell.

Clastogens and Mitotic Poisons - The use of chromosome studies to monitor possible environmental mutagens should not be limited to evaluations of chromosome-breaking or "clastogenic" effects on cells arrested in metaphase. If the cells are analyzed without conventional pretreatment with mitosis-arresting agents or hypotonic solutions, abnormalities in the anaphase can be identified. These include multipolar mitoses, imperfect or unequal separation of chromosomes, and bridges interfering with reconstitution of the daughter nuclei. Some abnormalities may be detectable

only in cells recovering from the effects of a chemical. During exposure the affected cells may be totally blocked from entering mitosis. Thus, in evaluating the potential action of a chemical as a chromosomal mutagen the investigator must look for both clastogenic and antimitotic effects. The latter may be especially important in chemicals that do not induce point mutations.

The effectiveness of benzene as a mitotic poison has been amply demonstrated. Decrease in DNA synthesis has occurred in cultured human cells[2-4] and in bone marrow of rats and rabbits after treatment *in vivo*.[5-10] The total numbers of nucleated cells, and, in some cases, the mitotic indices have declined. Inhibition of cell proliferation has been shown most often by decrease in uptake of radioactive-labelled thymidine, a DNA precursor. Although these may not be the most sensitive of indices, they are clearly and directly relevant to cell survival and reproductive fitness. Furthermore, both numerical and structural chromosome aberrations have been described that could be interpreted as either toxic or mutational damage. These include loss or gain of parts of chromosomes, whole chromosomes, or chromosome sets, in addition to exchanges that result in morphologically aberrant chromosomes.

Anaphase studies on human cells have not yet been reported but are under way in several laboratories. Morishima and his colleagues have described appropriate conditions for testing human material.[11]

Cytogenetic Aberrations in Leukemia - The assumption that chromosomal mutation is etiologically important in the development of leukemia has been strengthened by observations of abnormalities in human leukemic cells. The close association of the Ph[1] translocation with chronic myelogenous leukemia is well-known,[12,13] and specific chromosomal abnormalities have been reported with other forms of leukemia.[14-16] These abnormalities appear to be specific to each disease entity, confined to the leukemic cells, and clonal (indicating a probable single-cell origin). Therefore, it is clearly important to investigate the actions of potential leukemogens with particular emphasis on their ability to cause site-specific chromosomal lesions. It is, however, even more likely that the initial damage caused by most carcinogens is nonspecific, causing a genetically more variable population of cells. This, in turn, increases the probability that an abnormal proliferative state will arise (or be selected).

Cytogenetic Studies of Animals

Studies of benzene effects have been conducted in many species, including rats, mice, rabbits, and newts. These

studies have included whole-animal exposures and effects of benzene on cells *in vitro*; they have been based on either acute or chronic and repeated exposures. The results of such studies are difficult to evaluate since they differ not only in the biologic end-point chosen, but also in species, routes of administration, and dosage. Since few of the studies have involved inhalation exposure, their relevance to problems of human disease may be questioned.

Unpublished studies by Wolman et al evaluated chromosomal findings in rats chronically exposed to 300 or 100 ppm benzene. Within 10 weeks there was a striking and persistent increase in chromosome breaks and aneuploidy (deviation from the normal diploid chromosome number) in the bone marrow of treated (300 ppm) animals. The increase following 100 ppm exposure was not as great and not of clear statistical significance.

Increased chromosome breakage in several species has been reported. Rats exposed to benzene subcutaneously over a period of 12 days showed highly significant increases in chromosome aberrations of bone marrow cells over untreated and toluene-treated controls.[17] Classification of both gaps and breaks as aberrations complicates interpretation of these findings (since gap rates vary more with the interpreter and with preparation) and inflates the aberration

rate. For example, although significant increases in aberrations were also found in the toluene-treated controls in this study, the benzene-treated group was the only one in which breaks were more common than gaps as aberrations. Exchange figures such as might result from abnormal repair after breakage (i.e. ring forms, translocations, and dicentrics) were rarely seen. Another, more acute exposure (2.0 ml benzene/kg body weight for 12 to 72 hours) in rats produced similar findings.[18] Increased numbers of chromatid breaks were found at almost every exposure interval, although the responses of individual animals varied considerably. Chronic exposure to injection of 0.2 mg/kg per day in rabbits (up to 18 weeks of treatment) also resulted in a high frequency of aberrations; since less than 15 percent of the aberrations reported were breaks, the significance of this study[9] is not established. Again, exchange figures, dicentrics, and hyperploid cells were rare. In each of these animal-exposure studies only a single dosage of benzene was used. Thus, although different exposure times in different species can induce increases in chromosome aberrations, there is no clear evidence for a dose-dependent response to benzene exposure. Furthermore, none of these studies presents data suggestive of mutational rather than toxic damage. Very few experiments have addressed the

question of direct interference with benzene-induced abnormalities and possible therapeutic routes.

Studies of dividing erythroblasts taken from the amphibian newt (Molge vulgaris L.)[19,20] are of particular interest because of their demonstration of anaphase abnormalities. Young animals injected with water-saturated solutions of benzene were bled 6 to 12 hours later and a drop of tail blood was used for coverslip culture. At the time of sampling, 38 percent of mitoses were arrested in late metaphase. Another 28 percent showed evident anaphase abnormalities, of which 20 percent were migration arrests, 3 percent were anomalies of numerical distribution, and 3 percent were anaphase bridges. The remaining 2 percent showed small subgroups of chromosome condensations outside the two poles of the newly forming nuclei. Observations over several hours showed that these mitotic abnormalities resulted in unequal nuclear divisions, polynucleated cells, and atypical nuclei. Prophase and early metaphase anomalies were never found at the doses used in these studies (up to 54 mg of benzene per animal).

Cytogenetic Studies of Man

Experiments - A few experimental observations have been made on the responses of cultured human cells to addition of benzene to the culture medium. Increased incidence of

breaks and gaps was observed in leukocytes (white blood cells) and cancerous cells after brief exposure to 1.1 or 2.2×10^{-3} M benzene in vitro.[2-4] At the higher dose a decrease in DNA synthesis interfered with clear correlation of dose to the incidence of breaks. These findings were considered to be toxic damage. Another experiment[21] in which peripheral blood lymphocytes stimulated by phytohemagglutinin (PHA) were exposed to benzene during 72-hour culture revealed both numerical and structural alterations in the treated cells. Aneuploidy was seven times more frequent in the treated populations than in controls, and chromosome breakage was seen in 11 percent of the treated cells as compared with 1 percent in the controls.

Chromosome Studies and Hematologic Disease - In contrast to the paucity of experimental data, there is an abundance of reports on chromosome studies in exposed populations and of case reports on leukemia patients. The case reports are particularly difficult to evaluate and compare. Some individuals were exposed to benzene vapor above permissible levels,[22] but in many cases the exposure levels were unknown. The total periods of exposure ranged from brief and acute to as long as 22 years.[23] The times between exposure and the development of disease or death also varied greatly. Most of all, the endpoints of disease were not

comparable. The various reports include diagnoses of acute intoxication,[22] death with massive bleeding and extramedullary hematopoiesis,[22] benzene leukemia,[23] acute myeloid luekemia,[24] acute erythroblastosis,[25] erythroleukemia,[26] acquired aplastic anemia,[27,28] acute lymphoblastic leukemia,[29] myelofibrosis, and chronic myelogenous leukemia.[30] Chromosome abnormalities have been present in industrial workers in association with hematologic pathology[21] or pancytopenia.[32]

Prior blood transfusions, the use of PHA-stimulated lymphocytes in some cases and bone marrow in others, and the lack of chromosome breakage rates in controls also hamper interpretation of the results of chromosome studies. Nevertheless, certain trends appear amid this mass of data. Additional chromosomes were identified in several cases,[22,23,29,33] and in two cases the additional chromosome was identified as a member of the C group. Both were cases of acute leukemia, in which additional C-group chromosomes - usually number 8 - are frequently found;[16] therefore this does not, as one reviewer suggested,[34] constitute evidence of benzene etiology. Persistence of abnormal chromosomes long after exposure and illness was also reported.[35] Tetraploidy or polyploidy was found in several instances.[23,25,27,28] Increased chromosome breakage was reported but not well-documented.

It is important to emphasize that the end stage of exposure to benzene was as variable in alterations of the karotype, or chromosome "package," as it was in clinical manifestations. Indeed, the karyotypic changes may well have reflected the disease state more than they reflected the (presumed) inducing agents.

Occupational Exposures - The clearest picture of the relationship between benzene exposure and chromosome changes emerges not from experimental studies but from studies of occupationally exposed workers. Tough and Court-Brown observed unstable* chromosome damage in cultured lymphocytes from workers exposed to benzene solvents.[36] They and their collaborators expanded the study[37] to include groups from three factories and sex-matched controls. The first group of 20 men had been exposed to benzene at factory A for periods of 1 to 20 years and were tested 2 or 3 years after exposure ended. The second group of 12 had worked periods of 6 to 25 years in areas where benzene was present (factory B), the exposure ending approximately 4 years prior to the study. The third group of 20 had worked for periods of 2 to 26 years in a closed distillation plant (factory C). In each instance controls were selected from nonexposed indi-

* Unstable aberrations include open breaks, fragments, ring and multicentric chromosomes, and exchange figures. Stable aberrations include deletions, translocations, inversions, trisomies, and other alterations of chromosome number.

viduals in the same factories. Available measurements of atmospheric benzene were 25 to 150 ppm in factories A and B and approximately 12 ppm in factory C. The results indicated significant increases in unstable aberrations in exposed workers from factory A, in both exposed workers and controls in factory B, and in neither group in factory C. Furthermore, the exposed workers at factory A were older than their controls, and the authors demonstrated significant increases in aberrations in the general population with increasing age.

Several other investigators have reported increases in chromosome breakage or in stable and unstable aberrations in healthy workers.[38-40] In one report[39] the atmospheric concentrations of benzene were less than 25 ppm. More compelling results were obtained by Forni and co-workers,[41] who compared data on 34 workers in a rotogravure plant with those of matched controls. The group of workers was subdivided; 10 individuals had been exposed to benzene for periods of 1 to 22 years (measurements of benzene in the plant during a brief single period ranged from 125 to 532 ppm). These 10, with the remaining 24 workers, were then exposed to toluene for periods up to 14 years at levels ranging up to 824 ppm. The age- and sex-matched controls had no history of exposure to either solvent. The findings

in workers exposed only to toluene were not significantly different from those in the controls, but the group that had been exposed to benzene showed increases in both stable and unstable chromosome aberrations ($p < 0.01$). Another study[42] of 25 subjects who had recovered from clinical "benzene hemopathy" indicated that increases in both types of aberrations persisted several years after cessation of exposure, although there was some decrease in the proportion of unstable aberrations. Average values of unstable abnormalities in the exposed group were 3 times greater than in the controls, and of stable chromosome abnormalities, 30 times greater.

Most of these industrial studies were systematic to some degree, including controls and statistical evaluations of results. These studies of workers from several European countries all present similar results; that is, statistically significant increases in both numerical and structural chromosome alterations in populations exposed to benzene. PHA-stimulated lymphocytes showed both stable and unstable chromosome changes in the absence of detectable alterations of the bone marrow, and aneuploidy or polyploidy was reported frequently. In studies where little or no clinical symptomatology resulted from exposure, there was considerable variation among individuals. For example, in Girard's[40]

and Forni's[41] studies a few individuals within each benzene-exposed group were responsible for the significantly higher chromosome breakage rates in the exposed populations. Moreover, it is clear that these changes persisted for many years after exposure, particularly in persons who showed clear evidence of clinical illness from benzene. The persistence of damage has been likened to that occurring after exposure to ionizing radiation. The few reported instances of abnormal clone formation[42,35] are important in terms of possible leukemogenesis. There is no correlation, however, between the degree or length of exposure to benzene, the clinical symptoms, and the persistence or extent of chromosomal aberrations.

Summary

The available documentation strongly suggests that chromosome breakage and rearrangement can result from exposure to benzene and that damage may persist in hematopoietic and lymphoid cells. The aberrations in human cells appear nonspecific; that is, they are random within the genome and unrelated to the aberrations associated with various forms of leukemia. A dose-dependent relationship between exposure to benzene and amount of chromosome damage has not been demonstrated. Evidence that benzene causes disturbance in DNA synthesis suggests that its mutagenic

action could involve interference with mitosis. Cytogenetic analysis of anaphase and postmitotic damage has not been evaluated adequately.

Theoretical considerations and some clinical observations suggest a relationship between chronic benzene exposure, chromosome damage, and leukemia. Chromosomally aberrant clones are typical of some but not all human leukemias, and aberrant cells and clones have been observed in individuals exposed to benzene who have later developed leukemia. Many authors have suggested that the lack of an observed dose-response relation in benzene-induced chromosome damage is due to variation in individual susceptibility. Some studies have recorded biological effects at (chronic) exposure levels below 25 ppm. The report of a recent international workshop on the toxicology of benzene has commented on this literature: "No dose-effect relationship has so far been demonstrated for benzene-induced chromosome aberrations. In workers chronically exposed to levels in the range of 5 to 25 ppm of benzene, both positive and negative reports involve small numbers of workers and confirmation of negative data is required on larger groups." Increased susceptibility to chemical clastogens has been found in human cancer syndromes that are genetically determined.[43] The variable response to benzene may be attributed

also to such possibilities as activation of virus, suppression of immune surveillance, or cocarcinogenic activity of other chemicals.

More detailed evaluation of the cytogenetic effects of benzene will require definitive data on dose/response relationships, relating the frequency and severity of chromosome damage to the amount and duration of benzene exposure. Both clastogenic and antimitotic measures of chromosomal mutagenicity should be evaluated. Benzene dosage should be correlated with clinical effects as well as with the various measures of chromosome damage. When an appropriate animal model becomes available, the evolution and sequence of chromosome changes with initiation and progression of leukemia may become clear.

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